Project title: LSC Living Therapeutic Materials

Project number: W32/2019

Executive Summary

The Leibniz Science Campus "Living Therapeutic Materials" (LTMs) is an interdisciplinary research initiative launched in July 2020 at the Saarland Campus. It brings together expertise from materials science, pharmacy, medicine, and bioinformatics to develop innovative self-replenishing drug delivery systems aimed at treating chronic diseases. These systems, known as Living Therapeutic Materials, are designed for long-term and personalized administration of high-value biopharmaceuticals.

Research in the first four years has focused on the combination of materials, organisms and processing methods to fabricate LTMs that can release drugs long-term while preventing the release of the organism. Bacteria have been programmed to synthesize different biomolecules (darobactin, ectoin, hyaluronic acid, cannabidiol) in response to different stimuli (heat, light, aspirin), thereby allowing control of pharmacokinetic profiles. Living microcapsules, membranes and core-shell threads have been fabricated to target different clinical scenarios. The moderate immune response observed in vitro assays in this funding period encourages us to proceed with vivo experiments in the next years. The therapeutic performance of the models is being tested in vitro. Modern bioanalytic and bioinformatics methods have been established and will be applied to understand the interactions and risks between the programmed bacteria and the host cells.

The LSC is a cooperative initiative from the Leibniz Institute for New Materials (INM), the Saarland University (USAAR), and the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). It involves 19 research groups, including two junior group leaders. Within the years 2020-24, it provided interdisciplinary training to 29 PhD students, (14 in kind students). Of these, 55% came from abroad, and 43% have successfully defended their PhDs in the field of LTMs. Following a successful renewal application, LifeMat entered its second four-year funding phase in October 2024.

1. Achievement of objectives and milestones

In agreement with the workplan of the LSC, we highlight the following **scientific** achievements during the LSC LifeMat-1:

<u>WP1&2: We demonstrated functional and safe encapsulation of biofactories in synthetic hydrogels long term.</u> By tailoring the mechanical and diffusion properties of the encapsulating materials, we could regulate biofactory growth inside the hydrogel, and drug release. We identified hydrogel composition windows and physical requirements necessary to maximize drug production and maintain a constant drug release profile from the LTM over weeks. Core-shell and multilayer hydrogel designs with tailored mechanics across the LTM thickness prevented outgrowth of the organisms for several months (Bhusari et al., 2024; Bhusari et al., 2023; Bhusari et al., 2022). Selected organisms expected to be compatible with the host microbiome were engineered as biofactories: **(i)** *Corynebacterium* strains for the production of hyaluronic acid or ectoine for ophthalmological use and of curcuminoid for the treatment of cervical neoplasia and cancer (Beganovic & Wittmann, 2024; Jungmann et al., 2022); **(ii)** *E. coli* strains for heterologous production of antimicrobial Darobactin A derivatives (Gross et al., 2021; Seyfert, Muller, et al., 2023; Seyfert, Porten, et al., 2023); **(iii)** *Streptomyces albus* to produce antituberculosis drugs (pamamycins) (Ahmed et al., 2020; Oberhauser et al., 2025); **(iv)** *Lactobacillus* strains to secrete anti-inflammatory peptides (I6P7 & KCF18) and anti-proteolytic Elafin in the cervix (Dey et al., 2023; Tadimarri et al., 2025). Different processing technologies (electrospinning, coaxial bioprinting, microfluidic encapsulation) were adapted to encapsulate biofactories in different hydrogels (Pluronic, Poly(vinylalcohol), PEG/Dextran) and

geometries (multilayer thin films, fibrillar membranes, core-shell microparticles or printed filaments with coaxial layers) adapted to target the eye, cervix, and lung.

WP3: LTMs with on-demand delivery have been realized. A unique advantage of biofactories is the possibility to integrate sensors and switches for on-demand therapeutic release. Switches for light- and heat-responsive bacterial activity have been transferred to *ClearColi* and probiotic *E. coli* Nissle 1917 biofactories (Basaran et al., 2023), Dhakane et al. 2023). Controlled release of the antibiotic darobactin (Dey et al., 2024), a pro-angiogenic vascular endothelial growth factor (VEGF)-mimetic protein and the flavonoid pinocembrin has been achieved in living hydrogels (Riedel et al., 2023). Novel genetic parts for the probiotic *L. plantarum,* for which the genetic toolbox is underdeveloped, have also been engineered, including heat- and sugar-responsive gene expression systems. These results have led to a patent application (Dey et al., 2023). Thermoplasmonic hydrogel composites were used to encapsulate *E. coli* and demonstrate NIR-regulated protein production (Basaran et al., 2023). These examples demonstrate the flexibility of LTMs for controlled delivery using different external triggers.

WP4: Medical scenarios where LTMs could represent a reasonable therapeutic alternative were identified and drive LTM development in the LSC. **(i)** *Living contact lenses to deliver biopharmaceutics to the eye surface:* first laboratory prototypes secrete hyaluronic acid for weeks to prevent and treat contact-lens associated dry eye disease (Puertas-Bartolome et al., 2024), 2 patents filed in 2023) and are cytocompatible. In vivo experiments to confirm biocompatibility are in progress. **(ii)** *LTMs for the control of chronic viral infections, inflammation and carcinogenesis in the cervix.* Biofactories releasing curcuminoids, antiinflammatory compounds and natural antiviral molecules have been programmed. Implantable living therapeutic membranes for therapeutic delivery in the cervix are in development. Organoid and organotypic 3D culture models of the cervix have been developed and will be used for testing performance of the LTMs. **(iii)** *LTMs to treat lung infections* Acute and chronic lung diseases (i.e. CODP, lung cancer, pneumonia, asthma) cause significant morbidity and mortality, while treatment options are sparse. In patient screening experiments, disease-specific microbiome alterations and relevant lung commensals secreting valuable therapeutics in the lung have been identified. Our bioinformaticians have analyzed the metagenome and identified disease-characteristic species, genes and pathways (Srikakulam et al., 2023). Coculture models including lung-on-a-chip microfluidic systems to study the interaction between microbiome and lung cells are established and will be applied to in vitro testing of the efficacy of LTMs (Brand et al., 2024).

WP5: Evaluation of host-LTMs interactions and safety of LTMs. In vitro studies of the immune response to LTM prototypes have provided encouraging results. Human peripheral blood mononuclear cells from healthy donors showed no significant activation when exposed to LTMs containing engineered *ClearColi*. Monocytes were not activated when exposed to the supernatants of hyaluronic releasing C glutamicum LTMs (Yanamandra et al., 2023). Advanced toxicity tests in chicken chorioallantoic membrane (CAM) and zebrafish-based assays are in development. In vivo experiments (mice models) to evaluate the crosstalk between the bacteria and host cells are in progress, including in vivo monitoring of biofactories by high-resolution ultrasound imaging in combination with bioluminescence/fluorescence imaging. LSC members have participated in a Scientific Advice Meeting with the EMA in spring 2025 to discuss the regulatory challenges of LTMs.

The LSC has established a consortium of 19 PIs and 15 early career researchers (14 in-kind) across the 3 partner institutions in the Campus that closely collaborate within 12 cooperative projects in the field of LTMs. A dedicated training program including (see section 2) provided exceptional opportunities to the involved scientist to acquire complementary knowledge and network with the LTM community. Two independent junior groups have successfully developed distinct profiles in the field of LTMs as part of the LSC LifeMat-1. Dr. Shrikrishnan Sankaran, PI at the LSC since 2020, genetically programs probiotic bacteria to sense external stimuli and regulate the production of therapeutic biomolecules in response. Dr. Sara Trujillo, Associated Researcher at LSC since 2022, develops advanced tissue and disease models to assess the risks of GMO containing LTMs for human health and the environment (Desai, Mekontso, et al., 2025; Desai, Sankaran, et al., 2025; Mekontso et al., 2025; Trujillo et al., 2023).

The LSC LifeMat achieved a positive evaluation and approval of the continuation proposal. In the second funding period, we will continue the development and prepare technology transfer of LTMs focusing on material/organism/technology combinations that were successful in LifeMat-1 (2020-24) and on clinical scenarios where the expertise within the LSC can have the highest impact in science, innovation and future patient benefit. We will work on the fundamental understanding of technical determinants of quality, efficacy and safety in LTMs (WP1, WP2), and the development of methodologies to quantitatively demonstrate the positive benefit-risk-balance in in vitro and animal experiments (WP3). This also considers demonstrating the environmental and cost benefits of zero-waste LTMs (WP4) in comparison with other drug production processes. We will develop assays and guidelines towards a regulatory roadmap for LTMs, currently not existent. We will acquire and grow in-house expertise in transfer and regulatory issues concerning LTMs as cell-based therapeutic products and train early career scientists in unconventional competences (i.e. upscaling, medical regulation, standard operation procedures) to become professionals with unique and highly demanding profiles. This will include new forms of consultation and cooperation with industrial partners and regulatory bodies. The financial commitment of the partners will be maintained for the next four years.
Regarding the funding period being reported personnel costs accounted for around 85% of WGL's total funding and the majority of project expenditure. In consequence, the expenditure incurred was almost 10% higher than the amount calculated in the application. Of the personnel funds spent, around 192 k€ was spent on project coordination, while the remaining 573 k€ was spent on PhD students' salaries. Expenditure on consumables for the PhD students' research projects also exceeded the original request, totalling around 45 k€. These additional requirements were offset by shifting funds from the ‘Travel’ and ‘Publications’ areas, enabling the financial requirements to be adjusted to the actual course of the project.

2. Activities and obstacles

In an internal process, PIs were invited in three consecutive calls to submit a one-page proposal to obtain funding for a PhD student (initially for 3 years). The steering committee of the LSC selected 12 projects for funding attending to the following criteria: scientific originality, collaborative focus, synergy to other projects, expected impact for the LSC development and feasibility of the workplan. Selected PIs committed to support the collaboratives projects with an additional PhD student funded by their own group (in-kind contribution). Additional PhD students and postdocs in the Campus working on LTMs are also associated to the LSC and participate in the activities.
The LSC offered an extensive interdisciplinary training program to the early career researchers including: **(i)** a biweekly literature club with contributions from the PhD students (03/21-03/22); **(ii)** Biweekly lectures related to LSC topics by the PIs and occasionally by external lecturers (2021-22); **(iii)** A dedicated Lecture on LTMs (1SWS) within the MSc academic program of the Natural Science Faculty; **(iv)** An annual one-day retreat with the PIs to present and discuss the progress of the projects; **(v)** Two one-day mentoring workshops about career development in the academia and in the industry. A full list of the events can be found in the table. We also organized: **(vi)** two 3-day Summer Schools on Engineered Living Materials in June 2022 and July 2023 and **(vii)** the International Conferences on Engineered Living Materials in the years 2020, 2021 (virtual), 2022 and 2024 (with 200 scientists from 14 countries attending in 2024).
The pandemic situation delayed the recruitment of young scientists from abroad. Therefore, the Consortium applied for a cost-neutral extension of the project duration to December 2024 to account for this delay and to be able to accomplish our goals in the projects. This measure was approved by the Leibniz Association.

3. Results and successes

The following contributions can be highlighted:

1) Fundamental understanding on the material properties sensed by bacteria to be considered for living therapeutic materials design: Adv Sci (Weinh). 2022
2) First review article describing the opportunities and future challenges of living materials in a medical context: *Nat Rev Mater* 2021
3) Engineering thermo-regulated darobactin expression in probiotic E. coli.: Chem Sci. 2021, J. Biol. Eng. 2024
4) Developed light-regulated pro-angiogenic LTM: Adv. Funct. Mater. 2023
5) Pioneer in vitro study of the response of primary immune cells to living therapeutic materials: Biomater Adv 2023
6) Developed an LTM that converts a low-cost food-derived molecule into a high-value flavonoid: Front. Bioeng. Biotech. 2023
7) Expanded the genetic programmability of a probiotic lactobacillus for robust production of therapeutic proteins: *Microb. Biotech. 2023*, *Microb. Cell Fact. 2024*, *Small 2025*
8) Analytics and bioinformatics tools for omics profiling: Bioinformatics. 2023
9) Biofactories for ophthalmology applications: Microb Cell Fact. 2022
10) Innovative technology for self-lubricating living contact lenses reported: Adv Mater 2024. (DV MATER) and IP protected by two patent applications
11) 40 reviewed publications from LSC work (June 2020-2025), 65% published in the last two years (Figure 1A). 17 LSC PhD students are co-author of these publications. 43% include the collaboration of at least two PIs of the LSC (Figure 1B).

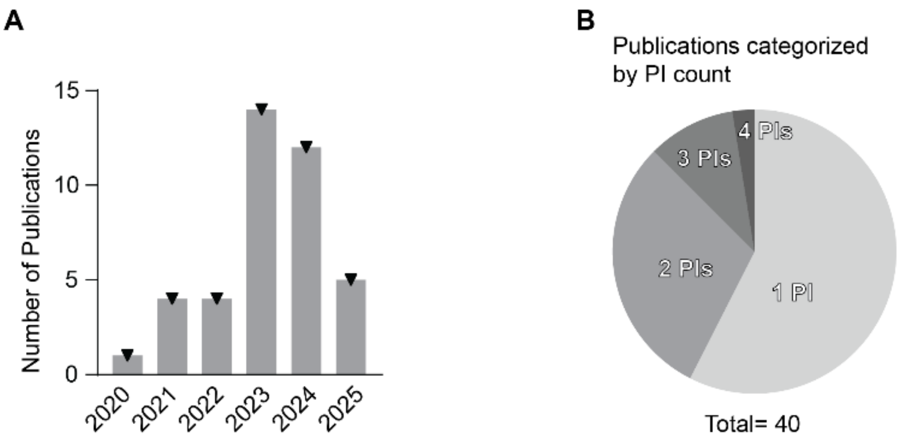

Figure 1: analysis of LSC related publications

The LSC is very visible through its webpage (https://www.lsclifemat.de) and social media feeds (@LSCLifeMat), LSC members are part of the international Slack group on Living Materials. We published several press releases and presented our work at the Open-door day of the Saarland University (2022, 2023, 2024).
The partner institutions have widened their commitment to LTMs beyond the plans in the original proposal. One important structural measure was the decision to co-fund additional Junior Groups to reinforce the work in the LSC (see section 1).
The visibility of the LSC contributed to the establishment of the Nachwuchsakademie 'Engineered Living Materials' and the SPP 2451 'Engineered Living Materials with Adaptable Functions' in 2024, both coordinated by the Speaker of the LSC.

4. Equal opportunities, career development and internationalisation

15 PhD positions were funded by the LSC (3 HIPS, 5 INM, 7 USAAR). The average contract duration was 3 years and 5 months. Additional 14 PhD students are associated to the LSC as in-kind contribution. 55% PhD students came from abroad and 55% are female scientists. To date, 13 students (43%) have completed their doctorate. 11 more students (38%) are expected to complete their doctorates in 2025. 7 Alumni of the LSC are postdoctoral researchers in other institutions, and 3 have transitioned to the industry. The students participated in a structured training program designed to introduce fundamental concepts in engineered living materials and living therapeutic materials, and to build expertise in this field (see 2).

5. Structures and collaboration

The LSC members share joint, collaborative topics and facilities. Infrastructure in all three institutions is available to all partners through an existing agreement. External cooperations are performed at individual member level and have not required changes to the existing agreement. Regular seminars and meetings are organized by the LSC coordinators to support scientific exchange and alignment to LSC goals.

6. Quality assurance

The steering committee of the LSC monitors the progress of the work in the LSC. The LSC counts on an international Scientific Advisory Board (SAB) with 6 members from the academy, industry and regulatory bodies. The first meeting took place in 2022. A second meeting is planned for November 2025. LSC members are trained in the rules of the Good Research Praxis and FAIR data principles at their own institutions. In vivo experiments planned within the LSC are approved by the local animal protection committee and executed in accordance with the European legislation on the protection of animals (Directive 2010/63/EU) and the NIH Guidelines on the Care and Use of Laboratory Animals (NIH publication #85-23 Rev. 1985).

7. Additional resources

The LSC's total budget for four years is 4.040 k€. The Leibniz Association provides financial support of 900 k€ and the Saarland state co-funds it with 400 k€.
The partners' internal contributions are distributed as follows: INM (1.020 k€), HIPS (540 k€) and Saarland University (1.180 k€). This sum includes in-kind contributions: 600 k€ from INM, 320 k€ from HIPS and 960 k€ from Saarland University, totalling 1.880 k€. Of this, 1.562 k€ was allocated to scientific staff (565 k€ for INM, 794 k€ for UdS and 203 k€ for HIPS), 62,5 k€ to non-scientific staff (12,5 k€ for UdS and 50 k€ for HIPS), 250 k€ to consumables and travel (29,5 k€ for INM, 153,5 k€ for UdS and 67 k€ for HIPS) and 5 k€ to investments (INM). Personnel funds totalled around 86,4% of the partners' in-kind contributions, 83,1% of which were allocated to young academic staff.
In addition to the in-kind services, the partners' own contribution included a fresh-money share totalling 860 k€, divided between 420 k€ for INM, 220 k€ each for UdS and HIPS. These funds relate exclusively to scientific staff and total 815 k€ (420 k€ for INM, 190 k€ for UdS and 205 k€ for HIPS), corresponding to a share of around 94.8%. The remaining expenditure totalled 45 k€ for consumables (UdS 30 k€, HIPS 15 k€).
Co-financing from Saarland was divided into around 330 k€ for personnel funds (UdS) and 70 k€ for investments (INM).

8. Outlook

The work in the LSC will continue along with the work packages defined in the continuation proposal, with a stronger focus on device testing and demonstration of performance and safety in close-to-application scenarios (in vitro and animal experiments) to prepare for translation.

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